Monitoring to Verify Confinement

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plan of presentation

- monitoring context
- monitoring approaches
- monitoring and QEA
- monitor and respond strategies

monitoring context

- focus on confinement of PMP/PMI
  - corn as base of experience
  - crop-to-crop gene flow
  - integrity of food/feed supply
- confinement and public policy
- confinement concern
  - episodic release to food/feed supply
  - accumulation in breeders seed

confinement and public policy

- *rights-based criterion*
  - primary concern is not outcome, but process and allowed action
- *zero risk criterion*
  - “independent of benefits and costs, and of how big the risks are, eliminate, or do not allow the introduction of, the risk”

Morgan and Henrion. 1990. Uncertainty: A Guide to Quantitative Risk and Policy Analysis.

confinement state-of-the-art

- commercial seed supply shows 99+% trait purity

  UCS. 2004. Gone to Seed.

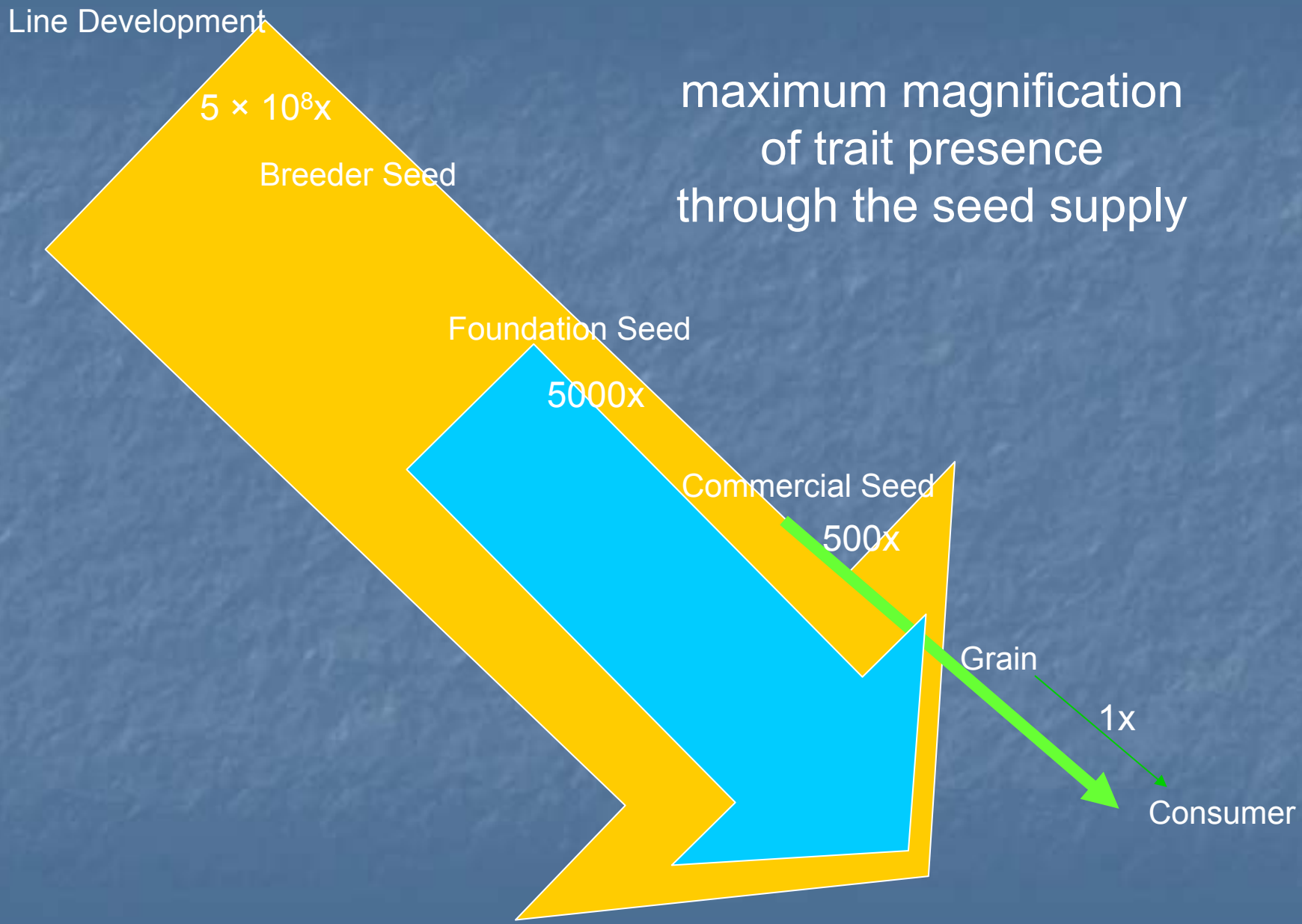
- Federal seed law mandates $\leq 10^{-3}$ frequency of unintended trait presence in foundation seed (99.9% pure)

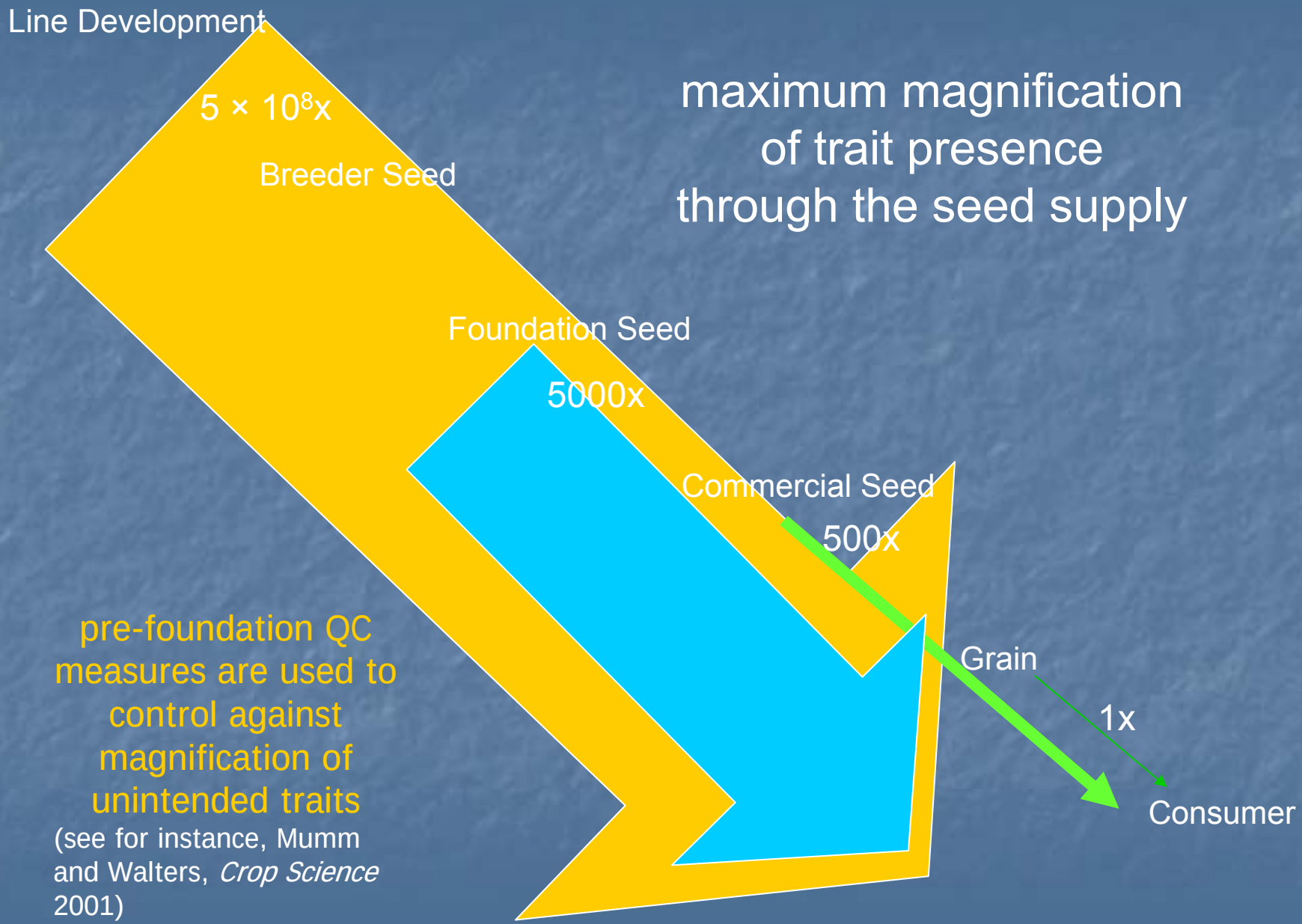
  ... and 99.5% purity for certified corn

- current practice meets or exceeds this standard

breeders' seed maintenance (pre-foundation seed)

- 30 seed each from 20 ears in unique rows
- 200 seed per ear
- 5 plants per row are hand pollinated
- 1 plant of the 5 is advanced to the next generation
- repeat for a second generation
- if OC frequency due to in-flow is 0.001,
- and no ability to detect,
- frequency for 1 contaminant seed to be retained in breeder's seed
  - 1 in 10^6, if intrusion is episodic in generation 1
    99.99+% pure
  - 1 in 250, if in generation 2 or recurring over generations
    99.6% pure
- if breeder is able to detect and rogue off-types
  ... likelihood of retention further reduced (10- to 10,000-fold)


Line Development
5 × 10^8x
Breeder Seed
maximum magnification
of trait presence
through the seed supply
Foundation Seed
5000x
Commercial Seed
500x
Grain
1x
Consumer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Breeder Seed
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Foundation Seed
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1x
Consumer
pre-foundation QC
measures are used to
control against
magnification of
unintended traits
(see for instance, Mumm
and Walters, *Crop Science*
2001)

monitoring breeders' seed

- limit recurrent presence of trait
- minimize potential for magnification through seed/grain channel
- focus monitoring efforts
  - in a given year, ca 10 acres breeder seed vs. 80 million acres grain

approaches to monitoring

- monitor for physical presence
- monitor for likelihood of escape
- monitor for process integrity

monitor for physical presence

- monitor pollen
  - indirect
  - pollen must be viable, reach a receptive plant, compete with receptor pollen, and effectively pollinate
- monitor outcrossing into receptor field of concern (or surrogate sentinel plot)
  - restricted analytical sensitivity
  - sample size constraints
  - high error rate (false positives/negatives)

zero tolerance (0% threshold) seed analysis perspective

- *exact definition = 0% lot impurity*
  - must test entire lot
- *hidden threshold = 0% in sample*
  - don't ask, don't tell
- *zero deviant plan = 0% positives in sample*
  - sensitive to false positives
  - high developer risk

monitor for physical presence

- detect and confirm 0.1% OC to a receptor
  - analyze 3000 seed and accept zero positives with 5% chance of accepting a field above 0.1%
- detect and confirm 0.01% OC in a receptor field
  - analyze 100 pools of 300 seed each and accept zero positives with 5% chance of accepting a field above 0.01%
  - analyze 50 pools of 320 seed each and accept zero positives with 20% chance of accepting a field above 0.01%
- detect and confirm at 0% OC to a receptor
  - analyze every seed

monitoring with sentinel plots

- detect and confirm decline over distance
- extrapolate to nearest field of concern

- limitations of approach
  - verification of model integrity
  - design and sampling intensity
  - extrapolation beyond data

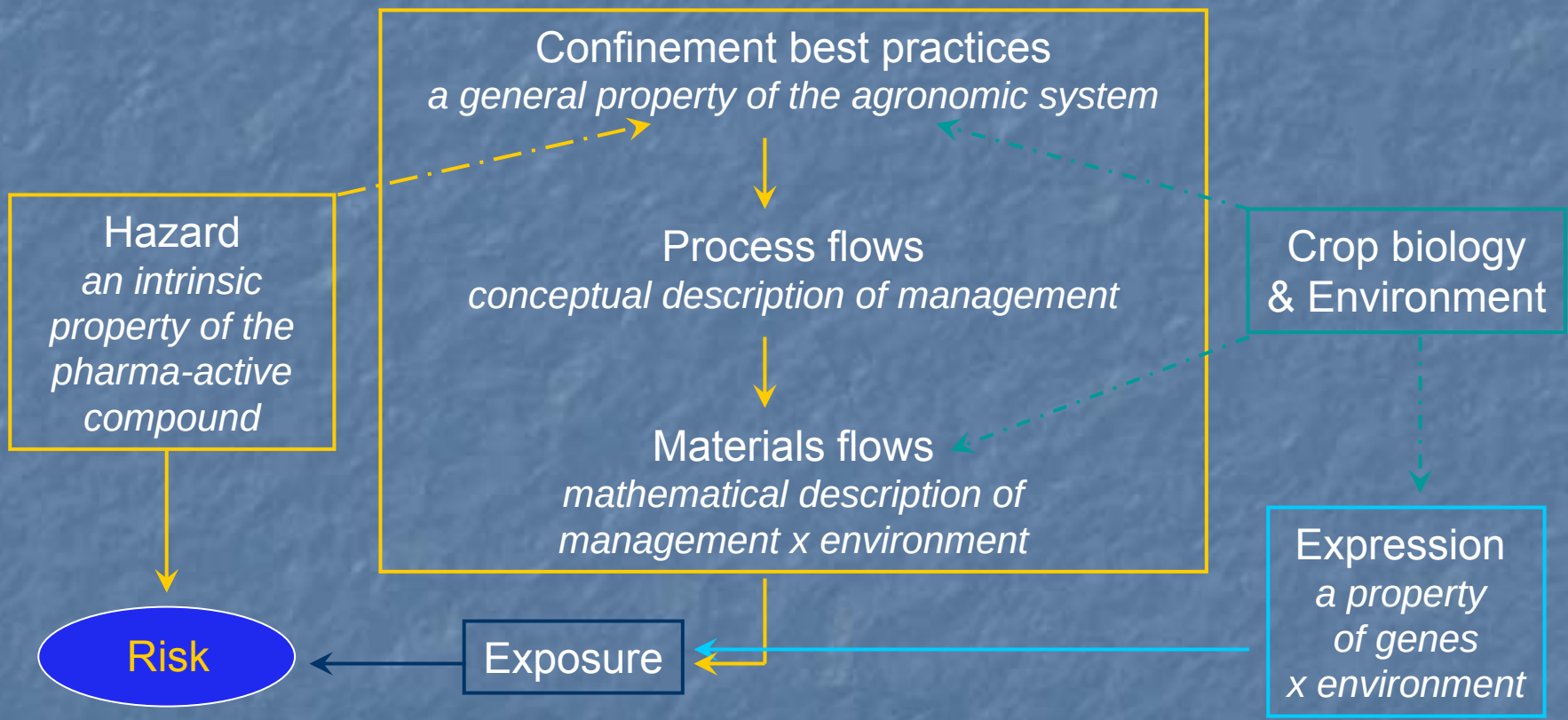
monitor for physical presence

- effective and reasonable for traits at 200m, but limited practicality at 1600 m
  - limit of detection
  - absence of validation data to verify extrapolation

monitor for process integrity

- design compliant processes
- use redundancy to address uncertainties
- monitor and audit process

BIGMAP Biopharma Confinement Project



QEA for process integrity

- describe process flows for confinement
- use QEA to
  - identify process uncertainties
  - identify critical control points
  - understand nature of magnitude of process failures

describe process flows for confinement

Pollen management

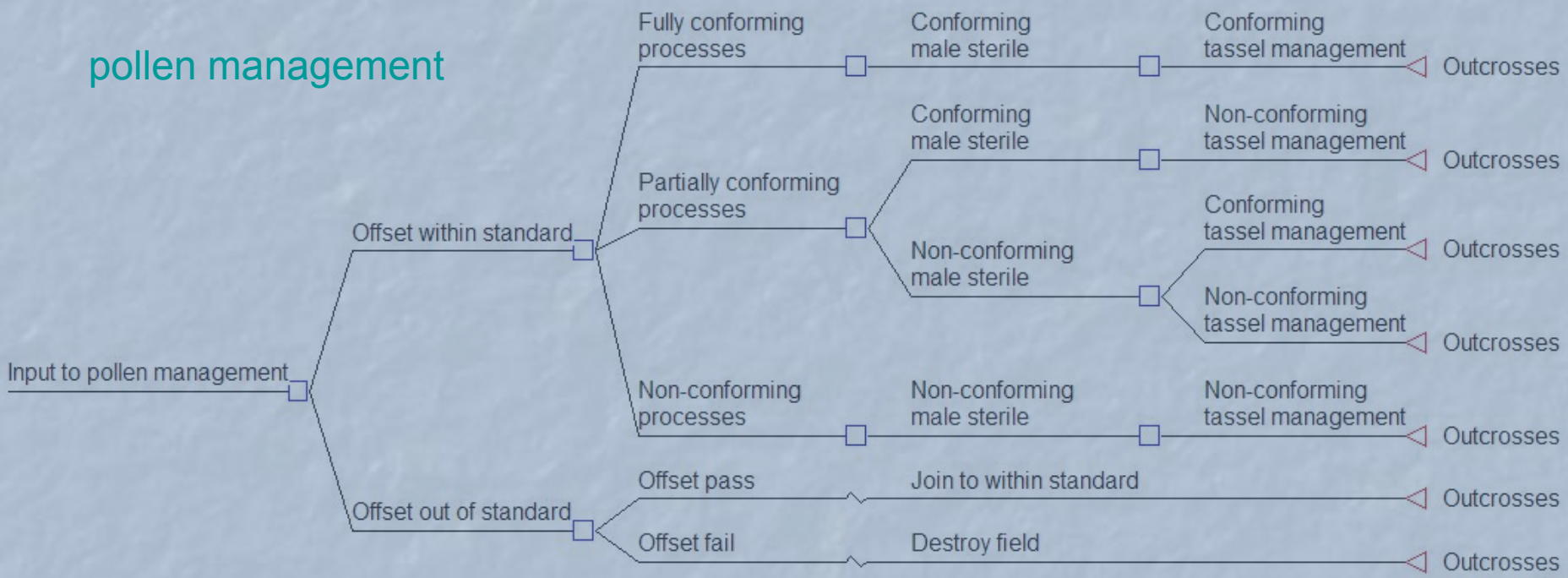
- Use trained personnel for pollen management sub-processes and cleaning
- Use approved procedures for pollen management sub-processes and cleaning
- Use dedicated and/or clean equipment for pollen management sub-processes
- Institute appropriate pollen controls
- Conduct pre-pollination identification and removal of off types/breakers
- Conduct post-pollination identification and removal of off types/breakers
- Confirm temporal and spatial isolation standards are achieved throughout the pollen shed interval
- Confirm overall pollen management sub-processes compliance

Harvest management

- Use trained personnel for harvest sub-processes and cleaning
- Use approved procedures for harvest sub-processes and cleaning
- Use dedicated and/or clean equipment for harvest sub-processes
- Conduct machine harvest in conformance to standards
- Conduct hand harvest operations to recover missed/dropped ears
- Document disposition of biogenic materials through harvest sub-processes
- Confirm overall harvest sub-processes compliance

identify process uncertainties

pollen management



understand process failures

relative number of fugitives	Deterministic result	Distributional result	
		50th percentile	90th percentile
	Outcrossing (to field at 1.61 km)		
Fully conforming	1	6	16
Partially conforming			
Male sterility system		59	166
Detasseling		50	100
Male sterility system + Detasseling		500	1,000
Non-conforming		1,467	15,333
	Harvest loss (left in field)		
Fully conforming	2,500	2,500	4,333
Partially conforming			
Combine		20,000	73,333
Ear picker		6,000	10,333
Non-conforming			
Combine		60,000	176,667
Ear picker		7,333	19,000
	Harvest loss (harvest mixing)		
Fully conforming	nil	nil	nil
Partially conforming			
Combine		1,200	
Ear picker		120	
Non-conforming		30,000	

monitor for likelihood of escape

- physical model for pollen flow/outcrossing
- site and confine to meet a predetermined confinement goal
- real time monitor key attributes of fugitive loss
  - wind speed, direction & timing; humidity; temperature
- identify departures from confinement goals

monitor and respond

- monitor, model, and identify departures from confinement goals in real time
- identify at-risk receptor fields
- segregate product from at-risk field prior to harvest (channel or destroy)

summary

- why monitor (PMPs/PMIs)?
  - independent of risk/benefit, do not allow introduction
- what to monitor?
  - line development and breeders' supply
  - minimize the possibility for recurrent presence
- how to monitor?
  - process integrity
- what does zero mean?
  - verification/validation of monitoring strategies/models
  - resolution of monitoring objective
- risk vs. zero tolerance?

BIGMAP

Biosafety Institute for Genetically Modified Agricultural Products
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  - seed supply and production
- Yuh-Yuan Shyy
  - database development
- Satish Rai
  - seed quality and analysis
- Manjit Misra
  - Director, BIGMAP

BIGMAP will provide science-based analysis of the risks and benefits of genetically modified plant and animal products. It will provide guidance and education to help safeguard consumers and the environment.